CALORIMETRY IN PHARMACEUTICAL RESEARCH AND DEVELOPMENT

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INTRODUCTION

Calorimetry is the measurement of energy changes within a material that are either manifested as exothermic (heat liberating) or endothermic (heat consuming) events (Table 1). Changes in energy (not absolute energies) are conventionally determined, and quantitative measurements may be made if the mass of the sample(s) is accurately known. Advanced and some custom-built instruments may permit the simultaneous monitoring of energetic changes along with other physicochemical events (e.g., changes in mass). These include, for example, microscopy Differential Scanning Calorimetry (microscopy-DSC) (1).

The most common applications of calorimetry in the pharmaceutical sciences are found in the "sub-fields" of DSC and Microcalorimetry. State-of-the-art DSC instruments and microcalorimeters are extremely sensitive and are powerful analytical tools for the pharmaceutical scientist.

DSC usually involves heating and/or cooling samples in a controlled manner, whereas microcalorimetry maintains a constant sample temperature. DSC instruments are considered to be part of the "Thermal Analysis" armamentarium; for additional information the reader should refer to the Thermal Analysis section of this book.

The beginning of this article gives a brief introduction into thermodynamics. A description of differential scanning calorimetry, which includes instrumentation, calibration, and applications, follows. A section on microcalorimetry is next, with a brief introduction into microcalorimetry, instrumentation, calibration, and applications. The article ends with a general comment on the regulatory aspects of calorimetry. A general description of the underlying physical or chemical transitions/reactions can be found in the section on DSC.

THERMODYNAMICS

The field of calorimetry relies on the principles of thermodynamics; the next section provides a brief overview of the general principles. References are provided for those who are unfamiliar with thermodynamics (2, 3).

A calorimeter consists of a container that is isolated from its exterior surroundings, where the heat exchange that occurs between the system and the environment can be measured. The "environment" is defined as the calorimeter and its contents, and the "system" is either a chemical reaction or physical change of state. The system can either absorb (endothermic) or lose energy (exothermic) to or from the environment. Exothermic changes will require the temperature of the environment to increase since it is the environment that is receiving the energy lost by the system. The energy of an isolated system remains constant and the energy exchange of the system must be equal but opposite in sign to the energy of the environment (First Law of Thermodynamics, Conservation of Energy). Endothermic changes in the system will involve a decrease in temperature of the environment since the environment is providing the energy absorbed by the system.

Based on the assumption that the system is closed, which is usually the case in differential scanning calorimetry and microcalorimetry, any reaction or change in state is independent of the path and can be subdivided into small reversible steps (Hess' Law of Summation) (2, 3). The First Law of Thermodynamics states that energy may neither be created nor destroyed. It defines the internal energy, dU, as the sum of the change in heat that has been transferred to the system, dq, and the work done on the system, dw.

$$dU = dq + dw \tag{1}$$

When operated at constant pressure, Equation (1) can be written in terms of the enthalpy, H. The total energy exchange between the system and the environment, the

Table 1 Common thermal events that can be detected using calorimetric techniques

Event	Example
Endothermic	
Fusion	Melting of drug substances; purity evaluations
Vaporization	Evaporation of liquid or semisolid excipients
Sublimation	Removal of frozen water during lyophilization
Desorption	Drying of wet granulated formulations
Desolvation	Removal of stoichiometric water from crystalline hydrates
Exothermic	
Crystallization	Solvent vapor induced crystallization of amorphous excipients
Precipitation	Formation of salt forms of drug substances
Solidification	Melt granulation with semisolid excipients
Adsorption	Solvent vapor sorption by drug substances
Chemisorption	
Solvation	Water vapor sorption by excipients
Curing of resins	Curing of polymeric packaging materials
Other	
Glass transition	Variation of glass transition temperature with water content
Relaxation of glasses	Enthalpic recovery of amorphous drug substance upon storage or annealing
Decomposition	Thermal decomposition of drug substance
Dissolution	Dissolving drug substance in dissolution media
Complexation	Complex formation between drug and cyclodextrin

enthalpy change (dH), is the sum of the change in internal energy of the system, dU, and the change in the amount of work, PV:

$$\mathrm{d}H = \mathrm{d}U + P\mathrm{d}V \quad \text{(at constant pressure)} \tag{2}$$

At zero net work and negligible change in volume (a close approximation for solids and liquids), the equation reduces to

$$(\mathrm{d}U)_\mathrm{p} = (\mathrm{d}H)_\mathrm{p} = (\mathrm{d}q)_\mathrm{p} \tag{3}$$

Thus, the enthalpy is effectively equal to the heat added or lost from the system, and changes in enthalpy can be measured directly in a calorimeter as dq (heat flow).

The heat exchange, dq, entering or exiting the system is equal to the change in enthalpy, dH, which is related to the heat capacity, Cp:

$$\mathrm{d}q = \mathrm{d}H = \int_{T_2}^{T_1} \mathrm{Cp}\,\mathrm{d}T \tag{4}$$

The increase in temperature of the system (from T_1 to T_2) is a function of its heat capacity. If Cp is large, then the transfer of a given amount of heat to a system results in only a small temperature increase.

DIFFERENTIAL SCANNING CALORIMETRY

During DSC, differences in heat flow between a sample and a reference are measured as a function of time and sample temperature. DSC analysis allows quantitative and qualitative information to be obtained about the physical and chemical changes that occur in a sample. DSCs are used extensively in the pharmaceutical industry to determine the melting points, purity, and glass transition temperatures of materials.

Instrumentation

Two types of differential thermal instruments, similar in practice but different in concept, are commercially available—differential thermal analyzers (DTA) and differential scanning calorimeters (DSC). Both techniques consist of a sample and reference pan, and provide quantitative and qualitative information on important physical phenomena of the sample (melting, purity, glass transition, etc.). These techniques differ both in sensitivity and the type of information that is recorded. DSC is a calorimetric technique where energy differences are measured as a function of time and temperature. DTA is solely a thermal technique where temperature differences and not energy are recorded as a function of time and temperature (Fig. 1a) (4, 5).

Two principal DSC designs are commercially available—power compensated DSC and heat flux DSC. The two instruments provide the same information but are fundamentally different. Power compensated DSCs heat the sample and reference material in separate furnaces while their temperatures are kept equal to one another (Fig. 1b). The difference in power required to "compensate" for equal temperature readings in both sample and reference pans are recorded as a function of sample temperature. Heat flux DSCs measure the difference in heat flow into the sample and reference as the temperature is changed. The differential heat flow to

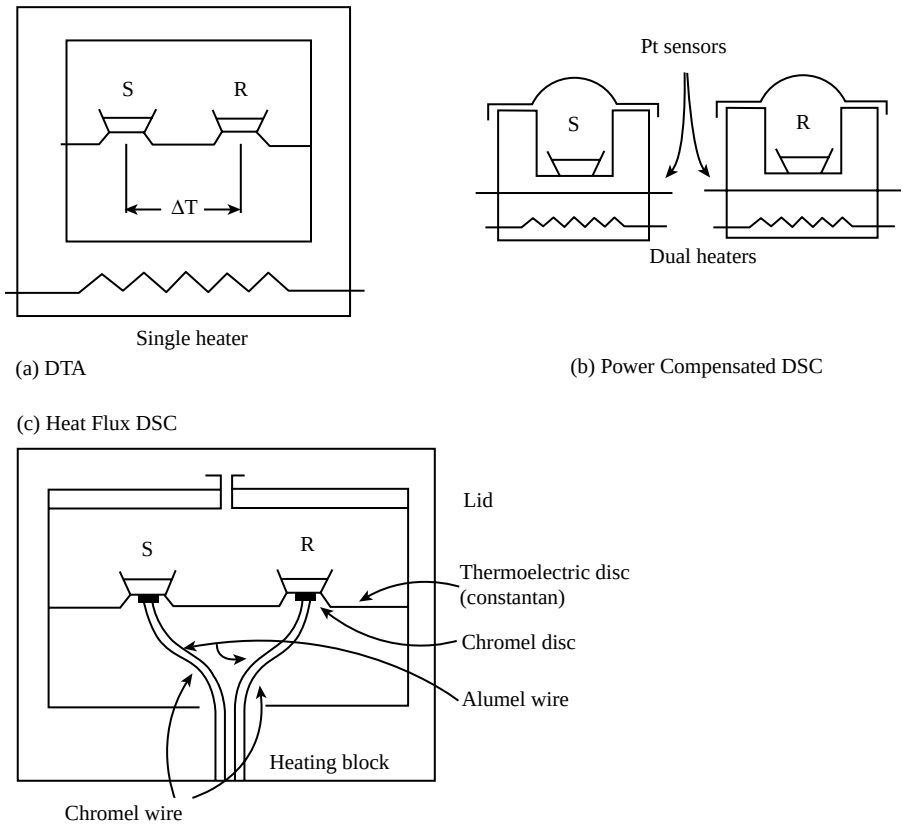

Fig. 1 Schematic diagrams of the (a) differential thermal analysis (DTA), (b) power-compensated differential scanning calorimeter, and (c) heat-flux differential scanning calorimeter cells (DSC) (adapted from DuPont Instruments Systems Brochure). (From Ref. 5.)

the sample and reference is monitored by chromel/constantan area thermocouples (Fig. 1c) (4, 5).

Modulated Differential Scanning Calorimetry/Dynamic Differential Scanning Calorimetry

Conventional DSC measures a sample's total heat flow. This total heat flow is comprised of a heat capacity component and a kinetic component (Eq. 5)

$$\begin{aligned}\text{total heat flow} &= \text{heat capacity component} \\ &\quad + \text{kinetic component} \\ \mathrm{d}q/\mathrm{d}t &= \mathrm{Cpd}T/\mathrm{d}t + f(T,t)\end{aligned} \tag{5}$$

where $\mathrm{d}q/\mathrm{d}t$ = heat flow, Cp = heat capacity, $\mathrm{d}T/\mathrm{d}t$ = temperature rate, $f(T, t)$ = heat flow from kinetic component as a function of temperature and time. Variations of conventional DSC have been used in order to extract additional information from these experiments. Two such techniques are Modulated Differential Scanning Calorimetry (MDSC) and Dynamic Differential Scanning Calorimetry (DDSC). Unlike conventional DSC, MDSC and DDSC determine the total heat flow, $\mathrm{d}q/\mathrm{d}t$, and the heat capacity component of heat flow, $C_p\ \mathrm{d}T/\mathrm{d}t$. Equation (5) then leads to the indirect determination of the kinetic component of heat flow, $f(T, t)$.

MDSC, developed by TA instruments (New Castle, DE), is based on the conventional heat flux DSC furnace design. The temperature programs differ in that a sinusoidal modulation is overlaid on the conventional linear heating or cooling rate to produce a continuously changing nonlinear sample temperature. This can be viewed as running two experiments at once. The first experiment consists of heating the sample at a constant linear rate to obtain the total heat flow much like conventional DSC. During the second experiment, the heat capacity component of the heat flow is obtained by continuously varying the temperature sinusoidally with a zero net temperature change during the course of the modulation. The experimental parameters may be optimized by modifying three variables—the average heating rate, the period of modulation, and the temperature

amplitude of modulation. Fourier Transformation of the modulated heat flow signal is used to calculate an average heat flow value, which is similar to the total heat flow obtained by conventional DSC. The heat capacity is determined by the ratio of the heat flow amplitude to the modulated heating rate amplitude. The heat capacity heat flow is then obtained by multiplying the heat capacity by the average heating rate. The kinetic component heat flow is obtained by the difference between the total heat flow and the heat capacity component.

$$dq/dt = Cp(dT/dt + A_T w^* \cdot \cos wt) + f'(t,T) + A_K(\sin wt) \quad (6)$$

where $(dT/dt + A_T w^* \cos wt)$ = measured heating rate, $f'(t,T)$ = kinetic response without temperature modulation, A_K = amplitude of kinetic response to temperature modulation.

DDSC provides heat capacity and kinetic component information differently from MDSC. The temperature program consists of an "Iso-Scan" whereby the traditional heating rate program is combined with several isothermal holds or a "Heat-Cool" program, which consists of combined heating and cooling temperature programs. The user selects the appropriate method depending on the type of experiment being performed (6). From the dynamic component of the sample response, the complex heat capacity can be calculated. The complex heat capacity, Cp*, is the vector sum of the storage, Cp′, and loss heat capacity, Cp″. It is generally the same as the storage heat capacity except in the melting region where heat losses dominate. The storage heat capacity is associated with molecular motions within the sample in a manner similar to the storage modulus in dynamic mechanical measurements. The out-of-phase component, the loss heat capacity, Cp″ is associated with the dissipative properties of the material. The loss heat capacity is out-of-phase with the temperature change because heat flow has resulted in molecular structural changes in the material. The loss tangent is the ratio of the loss heat capacity to the storage capacity and is a measure of the relative importance of each component (6).

$$Cp^* = Cp' + Cp'' \quad (7)$$

where Cp^* = complex heat capacity, Cp′ = storage heat capacity, Cp″ = loss heat capacity. Some of the advantages and disadvantages of using MDSC are given in Table 2.

Sample Preparation and Calibration

DSC samples are generally analyzed in small metal pans that consist of inert or treated metals (aluminum, platinum, silver, stainless steel, etc.). Several pan configurations exist, such as open, pinhole, covered, or sealed. Reference pans should be made of the same material as the sample pan and in identical configurations. Typical DSC sample sizes are 3–5 mg for pharmaceutical materials. The material should completely cover the bottom of the pan to ensure good thermal contact. The pan should not be overfilled to prevent thermal lag from the bulk of the material to the sensor. Physically stable compounds that consist of large granular particles should be ground to reduce unwanted thermal effects. Accurate weights are imperative if quantitative data of the sample's energetic parameters are desired (5).

DSC is a scanning technique that usually is used for relative rather than absolute measurements. The meaningfulness of the results depends in the care taken in calibrating the instrument as close to the transition temperatures of interest as possible. The accuracy of any thermo-analytical instrument is strongly dependent on the

Table 2 Advantages and disadvantages of modulated DSC

Advantages	Disadvantages
Ability to differentiate overlapping transitions	More complex thermal lag effects
Increased resolution without loss of sensitivity	Not as precise linear heating rate
Measurement of heat capacity and heat flow in a single experiment	Many experimental parameters
Measurement of initial crystallinity	Not recommended for melting transitions.
Ability to study previous thermal history	Gives sample a complex thermal history
Ability to distinguish between reversible and nonreversible transitions	Sometimes difficult to interpret

use of high purity calibration standards. Well-defined standards are especially important when analyses are carried out using different instruments and at different times. In general, metal calibration standards, such as indium, tin, bismuth, and lead, are utilized due to ready availability and ease of use. Low melting metals, such as mercury and gallium, are used to a lesser extent because of toxicity and handling problems. Organic compounds have been recommended as standards when studying organic material to minimize differences in thermal conductivity, heat capacity, and heat of fusion (7–9). It is likely that metals will continue to be popular temperature and enthalpy standards due to availability and ease of use, and organic standards may be used predominantly at temperatures below 300 K (10).

DSC results are dependent on the calibration of the instrument, sample preparation, and sample configuration. Some researchers argue that power compensated DSCs need to be properly calibrated upon both heating and cooling at the same rates in order to maintain a high level of accuracy (11, 12). Standard procedures can be obtained from the American Society for Testing of Materials (ASTM). In addition, all results obtained by DSC are a function of the scanning rate used and should be reported with the scanning rate. The shape, the area of the transition or change of baseline, and particularly the temperature of the transition will be dependent on the scanning rate; it will move to higher temperatures with increasing heating rate. Increasing the scanning rate increases sensitivity, while decreasing the scanning rate increases resolution. To obtain thermal event temperatures close to the true thermodynamic value, slow scanning rates should be used (e.g., 1–5 K/min).

Definitions and Applications of DSC

The purpose of this section is to define the various parameters that are measured by DSC. The types of thermal events, exothermic or endothermic, that can be measured by DSC are reported in Table 1. The following sections will describe some of the more fundamental thermal events. Examples from the pharmaceutical field will be given to illustrate the techniques. The examples will be based on either single components, such as drug substance and bulk excipients, or on a mixture of components, such as physical blends of drugs and excipients, solid dispersions, formulated drugs after granulation, and/or compression.

Melting

Melting is a first order endothermic process by which the compound takes in a net quantity of heat (molar heat of fusion). Through DSC, melting can be seen as an endothermic peak (Fig. 2). The broadness of the peak defines the purity of the crystalline compound undergoing melting, with the less pure and less perfect smaller crystals melting first followed by melting of the purer larger crystals (5). The melting temperature is the temperature at which the 3-dimensionally ordered crystalline state changes to the disordered liquid state. It is defined either as an extrapolated melting temperature onset, T_e, obtained at the intersection of the extrapolated baseline prior to the transition with the extrapolated leading edge, or as the peak melting temperature, T_m. Other temperatures that describe the melting process are the onset of melting, T_o, and the extrapolated end of the transition (5).

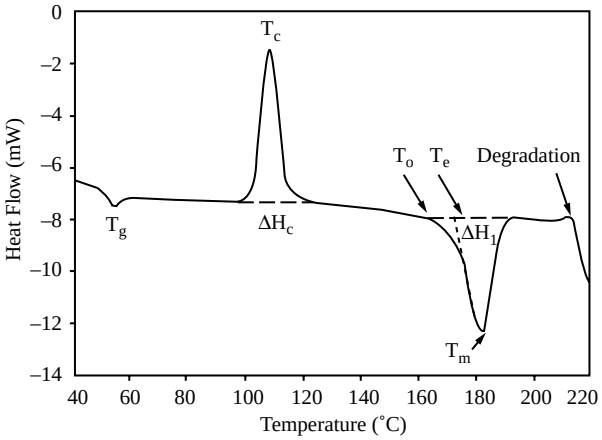

Fig. 2 DSC scan of sucrose showing the glass transition temperature, (T_g), recrystallization exotherm temperature (T_c) and enthalpy (ΔH_c), onset of melting (T_o), extrapolated melting onset (T_e), peak melting temperature (T_m) enthalpy of fusion (ΔH_f), and onset of degradation at 10 K/min. Endothermic transitions are shown down.

The enthalpy of fusion, ΔH_f, is obtained from the area of the endothermic transition. The area of the transition is affected by the selection of the baseline. The baseline is generally obtained by connecting the point at which the transition deviates from the baseline of the scan to where it rejoins the baseline after melting is completed. For some materials that undergo a significant change in heat capacity change on melting, other baseline approximations (such as a sigmoidal baseline) are used (5).

Purity

The purity of crystalline compounds can be calculated using the van't Hoff equation from the enthalpy of fusion and melting temperature obtained by DSC.

$$T_{s(i)} = T_e - RT_e^2 X/(\Delta H_f F_i) \quad (8)$$

where $T_{s(i)}$ is the sample temperature at equilibrium corrected for thermal lag effects (K), T_e is the melting temperature of the pure compound (K), R is the gas constant (8.314 J/mol/K), X is the molar fraction of impurity, ΔH_f is the enthalpy of fusion of the pure compound (J/mol), and$F$ is the fraction of the sample that is molten at $T_{s(i)}$. The melted fraction is equal to the area of the section melted (A_i) divided by the total area of the melting endotherm (A_T) as shown in Fig. 3. The melting depression, $(T_e - T_{s(i)})$ is equal to the slope, $(RT_e^2/\Delta H_f)X$, of the straight line obtained when $T_{s(i)}$ is plotted as a function of $1/F_i$. The theoretical melting temperature is obtained on extrapolation to $1/F_i = 0$. A straight line may not be obtained due to thermal lag, sensitivity, lack of a eutectic point detection, and formation of solid solutions. In addition, a significant amount of material may have melted before a measurable heat flow is observed by DSC. As a result, a correction constant K_{corr} is added to the measured areas (each fraction) to correct the curvature of the plot of $T_{s(i)}$ as a function of $1/F_i$ (Eq. 9). The melting depression $(T_e - T_{s(i)})$ is then obtained when $F_i = 1$ (5).

$$1/F_i = (A_T + K_{corr})/(A_i + K_{corr}) \quad (9)$$

It is necessary that the melting curve is obtained with a calibrated DSC using small samples (1–3 mg) and slow scanning speeds (<5 K/min, preferably 2 K/min). The purity of a compound should be determined at several scanning speeds to ensure that the compound does not undergo any solid-solid transitions, such as polymorphic conversion or degradation (5).

The three advantages of obtaining purity by DSC are: 1) its speed of measurement; 2) the type of impurity does not have to be known; and 3) a minimal amount of sample is required. However, in the case of salts, excess base or acid is counted as an impurity.

DSC calculations of drug purity were used to assess the quality of progesterone and lipoic acid, and to define specifications for the drugs (13). The enantiomeric purity of (−)ephedrinium 2-naphthalenesulphonate has also been determined using DSC (14).

Crystallization

Crystallization can occur on cooling from the melt and/or heating above the glass transition temperature of amorphous materials. The temperature at which this occurs is the crystallization temperature, T_c. Through DSC, crystallization is observed as an exothermic transition with an enthalpy of crystallization, ΔH_c (Fig. 2). The energy released when the molecules, atoms, or ions organize into a 3D solid state is related to the crystal lattice energy. Some compounds can crystallize into different molecular arrangements called polymorphs, discussed later.

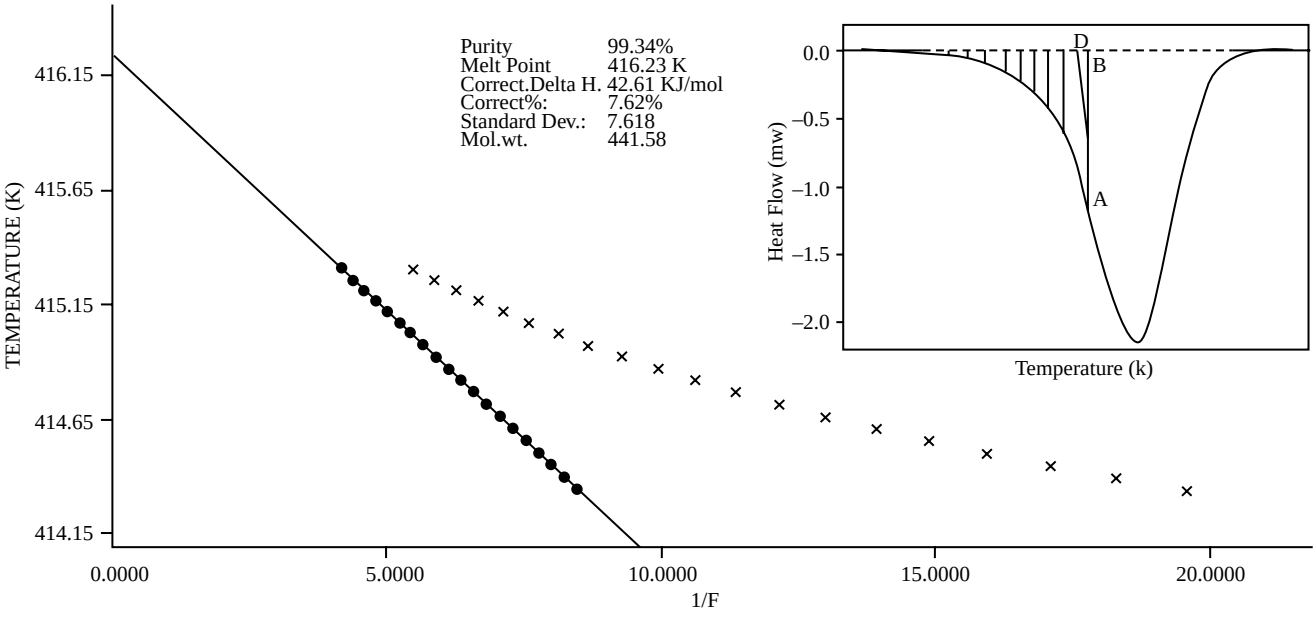

Fig. 3 DSC scan of drug substance divided into segments, A_i, for purity calculations of a compound of total enthalpy or area, A_T (inset, only a few segments are shown). The van't Hoff plot of the temperature of each segment as a function of $1/F = 1/(A_i/A_T)$, and with correction (straight line) for determination of purity of the drug substance.

Quantification of Crystallinity

The crystallinity of drugs and excipients before and after formulation processing can be determined using calorimetry. In some cases, crystalline compounds can convert during pharmaceutical processing to the amorphous form, which is a thermodynamically less stable form. Amorphous compounds consist of nonordered molecules (see the section on *Glass Transition*). This can have important implications for the chemical and physical stability of the formulations. The effect of grinding on the crystallinity of cefixime trihydrate was evaluated using DSC and other thermal techniques. The dehydration temperature of the ground sample decreased linearly with decreasing crystallinity (15).

An exothermic transition can sometimes be observed by DSC on crystallization of the amorphous form. This can be used to quantify the amorphous content of crystalline drugs. A calibration curve that consists of a plot of the enthalpy of crystallization as a function of crystalline content was used to determine if the lyophilized MK-0591 drug substance was completely amorphous or contained some crystalline compound (16).

Polymorphism

Polymorphs are crystalline compounds of the same molecular structure that have a different arrangement of molecules in the unit cell.[a] Polymorphs have the same chemical composition but have unique cell parameters. Therefore, polymorphs can have very different melting temperatures, densities, solubilities, chemical and physical stabilities, dissolution rates, and bioavailabilities (17).

Polymorphs are either enantiotropic or monotropic. Enantiotropic polymorphs have a thermodynamic conversion temperature where one form is more stable above this temperature while the other is more stable below. Processing the least stable form, dissolution/recrystallization, and certain storage conditions might cause enantiotropic polymorphs to later convert (5). If there is no conversion temperature below the melting temperatures of the polymorphic pair, then the different crystal forms are monotropic. That is, there is only one crystal form that is thermodynamically stable at all temperatures and pressures. Calorimetry can be used to determine which polymorph is the more stable form. DSC can provide accurate unambiguous melting temperatures and enthalpies of fusion. Based on the melting temperature and the enthalpy of fusion, the relative thermodynamic stability of the polymorphic pair can be determined [e.g., using the Heat of Fusion Rule (5)].

DSC and complimentary thermal techniques, such as temperature X-Ray powder diffraction, were used to determine the thermodynamic relationship of the six anhydrous polymorphs of tetracaine hydrochloride (17). The phase diagram of the polymorphic conversion of diflunisal in polyethylene glycol (PEG) 4000 solid dispersions was obtained as a function of polymer content (18).

[a]See *Polymorphism*: *Pharmaceutical Aspects*, page 2249.

Heat Capacity

Accurate heat capacity, Cp, measurements may be obtained by DSC under strict experimental conditions, which include the use of calibration standards of known heat capacity, such as sapphire, slow accurate heating rates (0.5–2.0 K/min), and similar sample and reference pan weights (19, 20). MDSC or DDSC also have been used to determine the heat capacity of several pharmaceutical materials (6, 21).

Glass Transition

By the use of various pharmaceutical manufacturing processes, (e.g., lyophilization or comminution techniques), drugs or excipients may be made amorphous. Amorphous compounds are defined by their lack of long-range molecular order and structural periodicity. Their high energy state is of great interest to the pharmaceutical industry as it can lead to fast dissolution rates and increased bioavailabilities. However, amorphous compounds are thermodynamically unstable, although depending on their glass transition temperature, they may be kinetically stable for extended times. Amorphous compounds are characterized by a glass transition, which by DSC is seen as an increase in heat capacity change (Fig. 2):

$$\Delta Cp = Cp_{liq} - Cp_{glass} \tag{10}$$

where Cp_{liq} is the heat capacity of the liquid, and Cp_{glass} is the heat capacity of the glassy phase. The glass transition temperature is measured either at its onset or midpoint as shown in Fig. 4. Structural relaxation can occur due to the restricted but finite mobility of the molecules below the glass transition. This gradual volume or enthalpy change is observed by DSC as an endothermic peak superimposed on the glass transition (Fig. 4), and this may lead to difficulties in interpretation of the transition. Modulated DSC can sometimes be used to separate the enthalpic overshoot from the glass transition temperature (5).

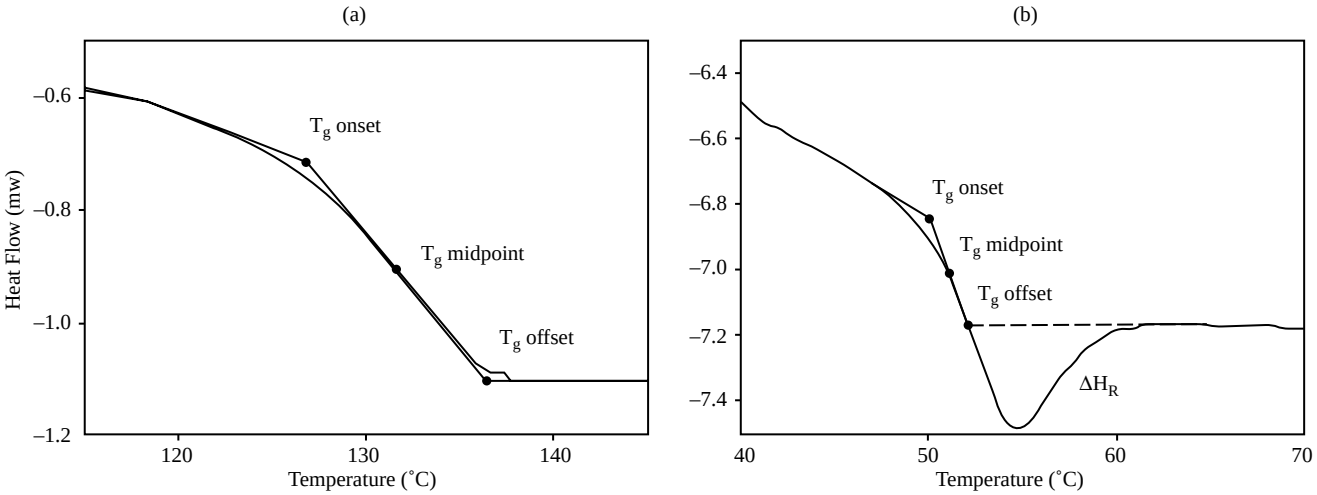

Fig. 4 (a) DSC scan of the glass transition temperature of a miscible blend of a MK-0591 with 10% PVP, showing the onset, midpoint, and offset glass transition temperatures. (b) DSC scan of the glass transition of sucrose with the enthalpic relaxation endotherm and enthalpy (ΔH_R). (From Ref. 5.)

Defining the glass transition temperature is important to the development of stable amorphous pharmaceutical materials. A leukotriene biosynthesis inhibitor, MK-0591, has been shown to be kinetically stable in the amorphous phase at normal storage temperatures if protected from moisture, due to its elevated glass transition temperature of 125°C (16). In lyophilized systems, a high T_g', defined as the apparent glass transition temperature and observed as the change of the heat capacity of the lyophilized formulations, is important to define the stability of such formulations (22–24). MDSC has been used to select optimal freeze-drying conditions to avoid cake collapse (25, 26).

The glass transition temperature of amorphous multicomponent mixtures can be used to determine the miscibility of the components. If the mixture is miscible, then a single glass transition temperature is usually obtained. Various equations can be used to predict the glass transition temperature of miscible mixtures. Examples include the Gordon-Taylor equation (Eq. 11) or the Fox-Flory equation (Eq. 12).

$$T_{g\,mix} = [w_1 T_{g1} + K w_2 T_{g2}](w_1 + K w_2); \quad K = \rho_1 T_{g1}/\rho_2 T_{g2} \tag{11}$$

$$1/T_{g\,mix} = 1/T_{g1} + 1/T_{g2} \tag{12}$$

where ρ_1 and ρ_2 are the densities of the two components and T_{g1} and T_{g2} are their respective glass transition temperatures (27).

Temperature Dependence of Molecular Motions in Amorphous Materials

A critical attribute that dictates the stability and performance of any amorphous material is the manner in which its rate of molecular motions (τ) varies with changing temperature (T) (i.e., $d\tau/dT$). At temperatures which are approximately 0–100 K above the calorimetric glass transition temperature (T_g), this property is known as the *fragility* of the material (27). Several workers have suggested that $d\tau/dT$ below T_g (in the nonequilibrium glassy state) is the most appropriate descriptor of amorphous pharmaceutical materials since this is the normal state for the storage and processing of such systems (27, 28). A simple graphical plot of τ versus T can be constructed at temperatures below T_g from the results of enthalpy relaxation experiments. These measurements can be performed using either a conventional DSC or microcalorimeter (28, 29). Alternate calorimetric methods of estimating $d\tau/dT$ at T_g have been described in the literature (30), but the applicability of these methods to pharmaceutical materials has not yet been clearly demonstrated (31).

Degradation, Decomposition, Stability Determinations, and Drug-Excipient Compatibility

The degradation, decomposition, and stability of drugs or formulations can be determined by DSC or microcalorimetry. The advantages of the techniques are their speed of measurement and the small amounts of sample required.

At times, interpretation of the results can be difficult, particularly when simultaneous reactions occur. Decomposition kinetics are generally determined using the Arrhenius equation. The samples are stored at elevated temperatures for known periods of time and analyzed by DSC. Alternatively, they can be held isothermally in the DSC at different temperatures, followed by scanning at heating rates sufficiently fast to avoid additional decomposition. A rate constant is calculated for each storage condition by plotting the logarithms of the areas of the transitions (e.g., the decomposition endotherm, etc.) as a function of time. The natural logarithm of the reaction rates, k, are then plotted as a function of $1/T$ as per the Arrhenius equation.

$$k = Ze^{-(E_a/RT)}; \quad \ln k = \ln Z - E_a/RT \tag{13}$$

where Z is the Arrhenius frequency or pre-exponential factor, E_a is the Arrhenius activation energy (J/mol) for the reaction, and R is the gas constant. The activation energy and pre-exponential factor are assumed to be constant and independent of temperature.

Alternatively, the reaction peak maxima may be determined at different heating rates (φ) and used to calculate the activation energy, assuming first order kinetics.

$$E_a = -2.19\,T\frac{\mathrm{d}\log\varphi}{\mathrm{d}(1/T)} \tag{14}$$

The energy of activation is obtained from the slope of the log of the heating rate (φ) as a function of $1/T$ (31). It is assumed that only one reaction occurs during the transition and that the peak maximum represents a point of constant conversion for each heating rate. The method cannot be used with compounds that decompose on melting or undergo isomerizations at the reaction temperature or any other simultaneous reaction (32). Some modification of DSC may be needed to determine the degradation kinetics of compounds under different environmental conditions.

DSC is often used for the rapid screening of excipients for drug-excipient compatibility studies. Certain assumptions have to be made, which include that the thermal properties of these mixtures are the sum of the individual components when there are no interactions between the components. The method does not take into consideration: 1) effects due to thermal conductivity (thermal lag effects); 2) mixing effects that can lower the purity of each component resulting in slightly broader, lower melting temperatures; or 3) sample geometry effects that result in variations in peak shapes and peak temperatures. In addition, reduction in enthalpies of fusion can occur as a result of the solubilization of the drug in molten excipients. This latter phenomenon can be used in part to determine the solubility in different molten excipients (5, 33).

Interactions with Water/Solvents, Hydrates

Water can have a significant impact on the physical and chemical stability of drugs. Water may be present as part of the crystalline lattice (hydrate), or it may be on the surface ("free") or more tightly incorporated ("bound"). The evaluation of the type of water present in a pharmaceutical material has been determined using subambient DSC (thermoporosimetry), such as in the case of magnesium stearate hydrates (34, 35), as well as thermogravimetric techniques. Free or surface water can crystallize and the melting enthalpy of this free water can be used to calculate the surface water content of compounds from the melting enthalpy of pure water (36). The state of water in HPMC gels with and without drugs, such as propanolol hydrochloride or diclofenac, was determined in this way by DSC (37).

MICROCALORIMETRY

Microcalorimetry is used to monitor thermal changes associated with physical and/or chemical events that do not require heating or cooling for their initiation. Such events include dissolution, precipitation, reaction, and crystallization. In a typical microcalorimetry experiment, these events are "triggered" in a controlled manner by mixing two pre-equilibrated and separate phases (e.g., water vapor and amorphous drug, solvent and crystalline drug, or protein and carbohydrate solutions). Microcalorimetry techniques are sometimes referred to by the processes that are monitored (e.g., immersion calorimetry, solution calorimetry, titration calorimetry, etc.) (38). Differential scanning calorimeters may be operated in isothermal mode; however, for highly accurate and reliable isothermal measurements, specially designed microcalorimeters are required.

Thermodynamics

Solution microcalorimetry may be used to determine the free energy of dissolution of a solid compound, which is particularly important in pharmaceutical research for dissolution studies and in the determination of the relative thermodynamic stability of polymorphs (38). The change in the Gibbs–Helmholtz free energy, ΔG_{sol}, on dissolution is

$$\Delta G_{sol} = -RT\ln K_{eq} \tag{15}$$

where T is the temperature (Kelvin, K), R is the gas constant ($8.314\,\mathrm{J\,mol^{-1}\,K^{-1}}$), and K_{eq} is the equilibrium constant for the change of the compound from the solid state to the dissolved liquid state. The equilibrium constant can be determined, at low concentrations, from the ratio of the concentration of the compound in the solution, or its solubility, to that in the solid state (where by definition, $[C]_{solid} = 1$).

$$K_{eq} = [C]_{soln}/[C]_{solid} = [C]_{soln} \tag{16}$$

The ΔH_{sol} is the enthalpy change that occurs on dissolution ofone mole of compound in a solvent. The solution microcalorimeter may be used to obtain the enthalpy of solution directly. The change of free energy can be calculated from the concentration using the enthalpy obtained. The change in the entropy of solution ΔS_{soln} can then be determined from the Gibbs–Helmholtz equation (2, 3).

Alternatively, the change in free energy of solution ΔG_{soln} can be calculated from the van't Hoff equation:

$$\delta(\Delta G_{soln}/T)/\delta T = -\Delta H/T^2 \tag{17}$$

It may be more accurate to obtain the enthalpy directly from microcalorimetry (39).

Instrumentation

The simplest type of nonscanning calorimeter is the isoperibol instrument (Fig. 5). In this type of calorimeter, a constant environment is maintained inside an insulated reaction vessel. Typically, a silvered dewar is used, and the interacting components (e.g., solvent and solute) are held in sub-containers. Liquid phases are usually stirred and the temperature is accurately recorded using a thermometer or thermocouple. At the start of the experiment, the reaction vessel is allowed to reach a steady state, and a baseline temperature or temperature drift is recorded. The interaction of interest is then initiated by permitting the two components to mix, and the resulting temperature increase from baseline is recorded. The system is calibrated by monitoring a standard reaction (e.g., neutralization of hydrochloric acid) or by applying a controlled amount of electrical energy via a heating coil. From this, the heat capacity of the system is determined, and the enthalpy change for any monitored process can be calculated from the observed temperature change. Isoperibol calorimeters can be easily constructed from their individual components, and several different instruments are commercially available at a modest cost.

Due to the high degree of accuracy and sensitivity that is required for pharmaceutical analysis, more sophisticated microcalorimeters are frequently used for studying

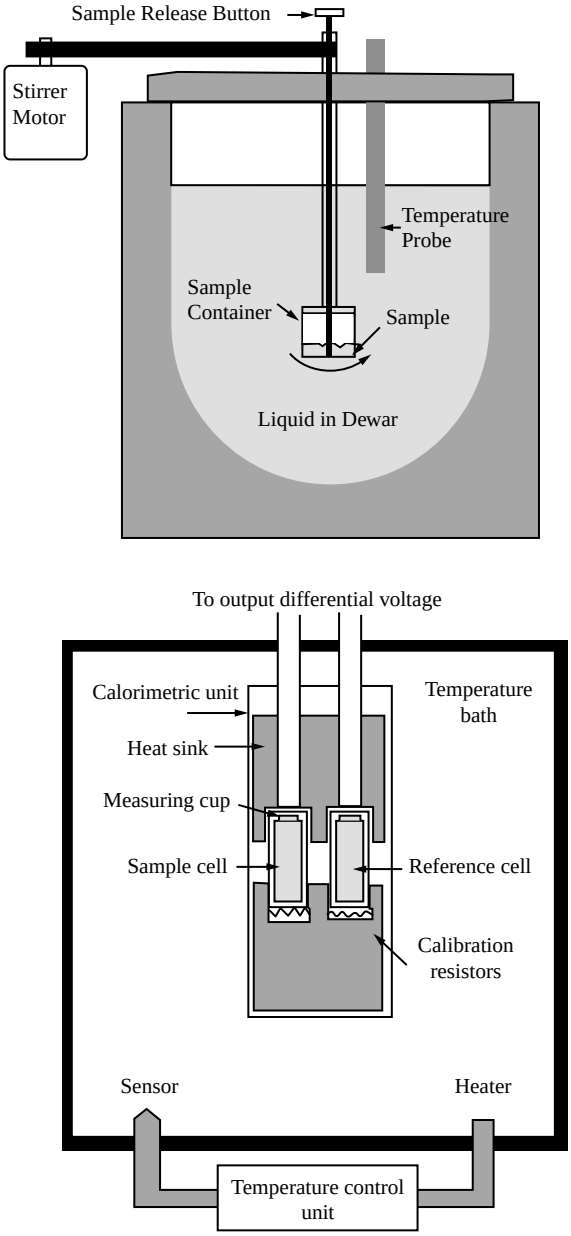

Fig. 5 Schematic diagram of an isoperibol calorimeter (top) and a thermal activity monitor (bottom).

pharmaceutical systems. An example of such an instrument is the Thermal Activity Monitor (TAM) manufactured by Thermometrics (Sweden). In this instrument, twin sample cells are used (one for the sample and one for a reference) to achieve greater signal stability and to minimize the effects of spurious thermal fluctuations (Fig. 5, bottom). The cells are housed in a constant temperature environment maintained via a sophisticated heater and water jacket system. Minor changes in sample heat flow are detected with relative ease using this

arrangement. The TAM is calibrated electrically, and the commercially available sample configurations allow the mixing of solids, liquids, and gases in various proportions. Controlled gas and liquid flow rates, and changing sample environments (e.g., relative humidities) can also be achieved with appropriate accessories. A major practical advantage of this type of calorimeter when compared with less sophisticated instruments is the small sample size requirement of only a few tens or hundreds of milligrams per determination. The TAM microcalorimeter has been used for detection and monitoring of crystallization events, sorption and desorption of organic and inorganic vapors, chemical reactions (drug degradation and drug interactions with excipients), molecular motions in amorphous pharmaceutical materials, ligand binding phenomena, microbiological growth, and the determination of solid heat capacities.

Sample Preparation and Calibration

Microcalorimeters are usually operated in a similar way irrespective of the source of the energy change that is being monitored. Specimens are pre-equilibrated at the desired measuring temperature for several hours and then introduced into the calorimeter chamber. After a short delay, the external stimulus is applied to trigger the event of interest, and then the energy that is liberated or consumed is measured. The reaction is isolated from the environment by a jacket, which serves as a thermal shield to minimize the absorption and emission of radiant heat (Fig. 5). Calibration of microcalorimeters is usually achieved by direct heating using an electric heating element (as mentioned previously). Extreme care is required to achieve consistent sample preparation and to maintain constant experimental procedures since the interpretation of results can be confused easily by experimental artifacts. Simultaneous thermal events of opposite sign (exothermic and endothermic) are quite common and may often confound the interpretation of data. In all experiments, an appropriate thermal reference is required since the energy changes that are measured are simply energy changes relative to the reference specimen. Common references include an empty sample container, or a sample container filled with an inert material which has a similar heat capacity and mass to the sample.

Definitions and Applications of Microcalorimetry

Microcalorimeters have found widespread use in the pharmaceutical sciences in recent years for applications as diverse as determining the degradation rate of drugs, estimating the strength of binding between proteins and receptor sites, and monitoring metabolic processes in microorganisms.

Hollenbeck used immersional calorimetry to investigate interactions between microcrystalline cellulose and water (39). Interactions of hydroxypropyl methylcellulose and cholestyramine with water also have been investigated by microcalorimetry (40, 41). In addition, the desorption of water from theophylline monohydrate has been investigated using microcalorimetric approaches (42). The properties of surfactants and surface active drugs in solution were studied by Attwood et al. (43) using calorimetry, while titration microcalorimetry has been utilized to elucidate the nature of specific interactions in several pharmaceutical polymer-surfactants systems (44, 45). A more unusual pharmaceutical use of microcalorimetry is to study energetic changes that occur during tablet compaction (46, 47). The compression calorimeter is a custom-made research instrument that appears to have many potential applications for the pharmaceutical scientist.

Microcalorimetry has proven to be a particularly useful tool to detect different levels of disorder in pharmaceutical materials (48). Pikal et al. used a solution calorimetry approach to measure differences in the crystallinity of a wide range of antibiotic samples (49). Gao and Rytting demonstrated the validity of this approach for a wider range of materials (50). Other workers have used elevated vapor pressures to trigger crystallization of disordered materials in the calorimeter and have been able to use the measured energy output to directly quantify the levels of disorder crystallinity in their samples (51).

Stability studies using microcalorimetry are widely reported in the literature (38, 52–54). Some authors have monitored exothermic degradation reactions over several days or weeks and have projected the degradation extent and rate over the shelf-life of the drug or drug product. Other authors have used microcalorimetry to monitor relaxation of amorphous pharmaceutical materials and have then calculated relaxation time constants from these data for use in shelf life predictions (29). The use of microcalorimetry for preformulation stability screening of a drug with potentially reactive excipients has also been described (55).

REGULATORY CONSIDERATIONS

Calorimetric methods are infrequently used for routine quality control purposes because of their nonspecific nature and relatively slow speed. However, data from calorimetry experiments are commonly presented in applications for

new product licenses and in support of patent applications. To ensure the integrity of all calorimetry data, normal procedures for good laboratory practices, standard operating procedures, appropriate calibration methods, and regular instrument servicing are necessary. The use of DSC for the measurement of transition temperatures and sample purity is described in the United States Pharmacopoeia, and standard procedures for DSC analyses are also suggested by the ASTM (100 Barr Harbor Dr., West Conshohocken, Pennsylvania 19428).

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